

CLAIMS

1. A microorganism which extracellularly secretes an unsaturated fatty acid-containing lipid as lipid vesicles encapsulating said lipid.

2. The <sup>isolated</sup> microorganism according to claim 1, wherein said unsaturated fatty acids are fatty acids having 18 carbons and two or more double bonds.

3. The microorganism according to claim 1 or 2 which is a filamentous fungus.

4. The microorganism according to claim 3 which is a microorganism belonging to genus *Mortierella*.

5. The microorganism according to claim 4 which is a microorganism belonging to genus *Mortierella* subgenus *Mortierella*.

6. The microorganism according to claim 5 which is a microorganism belonging to the species *alpina*.

7. The microorganism according to any one of claims 1 to 6 which has a property of forming lipid vesicles containing a lipid around the colonies when said microorganism is grown on a solid medium, and/or of making the culture liquid cloudy when said microorganism is cultured in a transparent liquid medium.

8. The microorganism according to any one of claims 1 to 7 which is obtained by artificially treating a microorganism having an ability to accumulate an unsaturated fatty acid-containing lipid in the cell.

9. The microorganism according to any one of claims 1 to 7 which is selected by artificially treating a microorganism having an ability of accumulating an unsaturated fatty acid-containing lipid in the cell, by culturing the obtained strains on a solid medium to select strains of which colonies are covered with lipid-containing lipid vesicles at the periphery, and then by selecting those strains that make the culture liquid cloudy when said microorganism is cultured in a transparent liquid medium.

10. The microorganism according to any one of

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Microorganism  
G. Mortierella  
elongata  
and mycelium  
alpina  
Sub A1

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Sub A2  
cont

Sub A2. 1 claims 1 to 9 which can be turned into a spheroplast or a protoplast.

sub B-27 11. A filamentous fungus having a property of  
extracellularly secreting a lipid containing unsaturated  
5 fatty acids.

Sub A3 7 12. The microorganism according to any one of claims 1 to 11 wherein said extracellularly secreted lipid is a lipid in which 50% or more is triglyceride.

10 13. The microorganism according to any one of  
claims 1 to 12 wherein said ~~unsaturated~~ fatty acids are  
arachidonic acid.

14. The microorganism according to claim 13 wherein said lipid contains 10% or more arachidonic acid relative to the total fatty acids.

15            15. Lipid vesicles encapsulating an unsaturated fatty acid-containing lipid.

16. The lipid vesicles according to claim 15 wherein said unsaturated fatty acids are unsaturated fatty acids having 18 or more carbons and two or more double bonds.

17. The lipid vesicles according to claim 15 or 16 wherein said lipid vesicles are produced by a microorganism.

25 18. Lipid vesicles encapsulating a lipid obtained from a culture liquid prepared by culturing the microorganism according to any one of claims 1 to 14 in a liquid medium.

19. The lipid vesicles according to any one of claims 15 to 18 which can be uniformly dispersed in water or a hydrophilic substance.

20. The lipid vesicles according to any one of claims 15 to 18 which stably retains the lipid encapsulated within said lipid vesicles against oxidation.

35 | 21. The lipid vesicles according to any one of  
claims 15 to 18 which can be separated by centrifugation.

22. The lipid vesicles according to any one of

claims 15 to 21 wherein the membrane of said lipid vesicles comprises sugar, protein, and lipid.

23. The lipid vesicles according to any one of claims 15 to 22 which has an average diameter of 0.2 to 10  $\mu$ m.

24. The lipid vesicles according to any one of claims 15 to 23 wherein the lipid encapsulated in said lipid vesicles is a lipid in which 50% or more is triglyceride.

25. A lipid isolated from the lipid vesicles according to any one of claims 15 to 24.

26. A food, a cosmetic, or an animal feed comprising the lipid vesicles according to any one of claims 15 to 24 added thereto.

27. The food according to claim 26 wherein the food comprising the lipid vesicles added thereto is a functional food, a nutrient supplement, formula for premature infants, modified milk for babies, a baby food, a food for pregnant women or a food for the aged people.

28. The food according to claim 26 wherein the foods to which the lipid vesicles have been added are beverages.

29. A food, a cosmetic, a pharmaceutical or an animal feed comprising the lipid according to claim 25 added thereto.

30. A method of producing lipid vesicles which method comprises culturing the microorganism according to any one of claims 1 to 14 in a liquid medium and then collecting the lipid vesicles encapsulating a lipid from the culture liquid.

31. A method of producing lipid vesicles which method comprises continuously culturing the microorganism according to any one of claims 1 to 14 in a liquid medium and then continuously collecting the lipid vesicles encapsulating a lipid from the culture liquid.

32. A method of producing a lipid which method comprises culturing the microorganism according to any

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one of claims 1 to 14 in a liquid medium, collecting lipid vesicles encapsulating a lipid from the culture liquid, and separating a lipid containing fatty acids from said lipid vesicles.

5 33. A method of producing unsaturated fatty acids which method comprises culturing the microorganism according to any one of claims 1 to 14 in a liquid medium, collecting lipid vesicles encapsulating a lipid from the culture liquid, separating the lipid containing  
10 fatty acids from said lipid vesicles, and isolating the unsaturated fatty acids from said lipid.

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15 34. A microorganism having a property of extracellularly secreting a lipid containing unsaturated fatty acids that have 18 carbons and three or more double bonds or 20 or more carbons and two or more double bonds.

20 35. A microorganism having a property of directly and extracellularly secreting a lipid containing unsaturated fatty acids that have 18 carbons and three or more double bonds or 20 or more carbons and two or more double bonds.

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25 36. The microorganism according to claim 34 or 35 which is a filamentous fungus.

30 37. The microorganism according to any one of claims 34 to 36 which has a property of forming lipid-containing lipid vesicles around the colonies thereof when said microorganism is grown on a solid medium, and/or of making the culture liquid cloudy when said microorganism is cultured in a transparent liquid medium.

35 38. The microorganism according to any one of claims 34 to 37 obtained by artificially treating a microorganism which has an ability of intracellularly accumulating a lipid containing fatty acids that have 18 carbons and three or more double bonds or 20 or more carbons and two or more double bonds.

39. The microorganism according to claim 35 or 36 obtained by artificially treating a microorganism which has an ability of intracellularly accumulating a lipid

5 containing fatty acids that have 18 carbons and three or more double bonds or 20 or more carbons and two or more double bonds, and by selecting, from the strains obtained, strains that make the culture liquid cloudy and then separates a lipid layer when cultured in a transparent liquid medium.

40. The microorganism according to any one of claims 34 to 39 which can be turned into a spheroplast or a protoplast.

10 41. The microorganism according to any one of claims 34 to 40 wherein said extracellularly secreted lipid is a lipid in which 50% or more is triglyceride.

15 42. A method of producing a lipid containing unsaturated fatty acids which method comprises culturing the microorganism according to any one of claims 34 to 41 in a liquid medium and collecting the lipid from the culture liquid.

20 43. A method of producing a lipid containing unsaturated fatty acids which method comprises continuously culturing the microorganism according to any one of claims 34 to 41 in a liquid medium and then continuously collecting the lipid from the culture liquid.

25 44. A screening method wherein a microorganism having an ability of extracellularly secreting a lipid containing unsaturated fatty acids using as an index the fact that when the microorganism is cultured using a transparent liquid medium the culture liquid becomes cloudy.

30 45. The screening method according to claim 44 wherein said unsaturated fatty acids have 18 carbons and three or more double bonds or 20 or more carbons and two or more double bonds.

35 46. The screening method according to claim 44 wherein said microorganism is a filamentous fungus.

47. A screening method wherein strains having a property of extracellularly secreting an unsaturated

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5 fatty acid-containing lipid are selected by artificially treating a microorganism having an ability to accumulate the unsaturated fatty acid-containing lipid in the cell, and by culturing the strains obtained on a solid medium to select strains of which colonies are covered with lipid-containing lipid vesicles at the periphery.

10 48. A screening method wherein strains having a property of extracellularly secreting an unsaturated fatty acid-containing lipid are selected by artificially treating a microorganism having an ability to accumulate the unsaturated fatty acid-containing lipid in the cell, by culturing the strains obtained on a solid medium to select strains of which colonies are covered with lipid-containing lipid vesicles at the periphery, and by  
15 further culturing the selected strains in a transparent liquid medium to select strains for which the culture liquid becomes cloudy.

20 49. The screening method according to claim 47 or 48 wherein said artificial manipulation is mutation treatment with N-methyl-N'-nitro-N-nitrosoguanidine (NTG).

25 50. The screening method according to claim 47 or 48 wherein said artificial manipulation is mutation treatment, gene manipulation, or cell fusion.

51. A microorganism selected by the screening method according to any one of claims 44 to 50.

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